

IN THE SPECIFICATION

Please insert the following new paragraph on page 1 at line 2.

This application is an RCE of application No. 09/645,321 filed August 25, 2000.

IN THE CLAIMS:

Please cancel claims 1-3.

Please amend claims 4, 5, 17 and 18 and add new claims 19-22 to read as follows. A marked-up copy of these claims showing the changes made thereto, is attached.

4. (Amended) A process for producing N-acetylneuraminic acid which comprises:

providing in an aqueous medium (i) a culture of a microorganism having N-acetylneuraminic acid aldolase activity or N-acetylneuraminic acid synthetase activity, or a treated matter of the culture, (ii) a culture of a microorganism capable of producing pyruvic acid or a treated matter of the culture when a microorganism having N-acetylneuraminic acid aldolase activity is used in (i) above, or a culture of a microorganism capable of producing phosphoenolpyruvic acid or a treated matter of the culture when a microorganism having N-acetylneuraminic acid synthetase activity is used in (i) above, (iii) N-acetylmannosamine which is produced by allowing a culture of a microorganism harboring DNA encoding N-acetylglucosamine 2-epimerase derived from a microorganism belonging to the genus *Synechocystis*, or a treated matter of the culture and N-acetylglucosamine to be present in an aqueous medium to form and accumulate N-acetylmannosamine in the aqueous medium, and (iv) an energy source which is necessary

for the formation of a pyruvic acid or phosphoenolpyruvic acid;

allowing N-acetylneuraminic acid to form and accumulate in the aqueous medium; and

recovering N-acetylneuraminic acid from the aqueous medium.

5. (Amended) A process, for producing N-acetylneuraminic acid which comprises:

(12
cont.)
providing in aqueous medium (i) a culture of a microorganism having N-acetylneuraminic acid aldolase activity or N-acetylneuraminic acid synthetase activity, or a treated matter of the culture, (ii) a culture of a microorganism capable of producing pyruvic acid or a treated matter of the culture when a microorganism having N-acetylneuraminic acid aldolase activity is used in (i) above, or a culture of a microorganism capable of producing phosphoenolpyruvic acid or a treated matter of the culture when a microorganism having N-acetylneuraminic acid synthetase activity is used in (i) above, (iii) N-acetylmannosamine which is produced by allowing a culture of a microorganism harboring DNA encoding N-acetylglucosamine 2-epimerase or a treated matter of the culture and N-acetylglucosamine to be present in an aqueous medium to form and accumulate N-acetylmannosamine in the aqueous medium, wherein said DNA encoding N-acetylglucosamine 2-epimerase is selected from the group consisting of: (a) DNA encoding a protein having the amino acid sequence shown in SEQ ID NO: 1; and (b) DNA having the nucleotide sequence shown in SEQ ID NO: 2, and (iv) an energy source which is necessary for the formation of pyruvic acid or phosphoenolpyruvic acid;

allowing N-acetylneuraminic acid to form and accumulate in the aqueous medium; and

recovering N-acetylneuraminic acid from the aqueous medium.

17. (Amended) A process for producing N-acetylneuraminic acid which comprises:

C³ providing in an aqueous medium (i) a culture of a microorganism having N-acetylneuraminic acid adolase or a treated matter of the culture, (ii) a culture of a microorganism capable of producing pyruvic acid or a treated matter of the culture, (iii) N-acetylmannosamine produced by allowing a culture of a microorganism carrying DNA encoding N-acetylglucosamine 2-epimerase activity selected from the group consisting of (a) DNA encoding a protein having the amino acid sequence shown in SEQ ID NO: 1; and (b) DNA having the nucleotide sequence shown in SEQ ID NO: 2 or a treated matter of the culture and N-acetylglucosamine to be present in an aqueous medium to form and accumulate N-acetylmannosamine in the aqueous medium, and (iv) an energy source which is necessary for the formation of pyruvic acid;

allowing N-acetylneuraminic acid to form and accumulate in the aqueous medium; and

recovering N-acetylneuraminic acid from the aqueous medium.

18. (Amended) A process for producing N-acetylneuraminic acid which comprises:

providing in an aqueous medium (i) a culture of a microorganism having N-acetylneuraminic acid synthetase activity or a treated matter of the culture, (ii) a culture of a microorganism capable of producing phosphoenolpyruvic acid or a treated matter of the culture, (iii) N-acetylmannosamine produced by allowing a culture of a microorganism carrying DNA encoding N-acetylglucosamine 2-epimerase activity selected from the group consisting of (a) DNA encoding a protein having the amino acid sequence shown in SEQ ID NO: 1; and (b) DNA having the nucleotide sequence shown in SEQ ID NO: 2 or a treated matter of the culture and N-acetylglucosamine to be present in an

aqueous medium to form and accumulate N-acetylmannosamine in the aqueous medium ,
and (iv) an energy source which is necessary for the formation of phosphoenolpyruvic acid;

allowing N-acetylneuraminic acid to form and accumulate in the
aqueous medium; and

recovering N-acetylneuraminic acid from the aqueous medium.

19. (New) A process for producing N-acetylneuraminic acid which
comprises:

providing in an aqueous medium (i) a culture of a microorganism
having N-acetylneuraminic acid adolase or a treated matter of the culture, (ii) a culture of a
microorganism capable of producing pyruvic acid or a treated matter of the culture, (iii) a
microorganism having N-acetylglucosamine 2-epimerase activity which carries DNA
encoding N-acetylglucosamine 2-epimerase derived from a microorganism belonging to the
genus *Synechocystis*, and (iv) an energy source which is necessary for the formation of
pyruvic acid;

allowing N-acetylneuraminic acid to form and accumulate in the
aqueous medium; and

recovering N-acetylneuraminic acid from the aqueous medium.

20. (New) A process for producing N-acetylneuraminic acid which
comprises:

providing in an aqueous medium (i) a culture of a microorganism
having N-acetylneuraminic acid synthetase activity or a treated matter of the culture, (ii) a
culture of a microorganism capable of producing phosphoenolpyruvic acid or a treated
matter of the culture, (iii) a microorganism having N-acetylglucosamine 2-epimerase
activity which carries a DNA encoding N-acetylglucosamine 2-epimerase derived from a

microorganism belonging to the genus *Synechocystis*, and (iv) an energy source which is necessary for the formation of phosphoenolpyruvic acid;

allowing N-acetylneuraminic acid to form and accumulate in the aqueous medium; and

recovering N-acetylneuraminic acid from the aqueous medium.

21. (New) The process according to claim 17, wherein said culture of a microorganism carrying DNA encoding N-acetylglucosamine 2-epimerase activity or treated matter thereof is copresent with said cultures of a microorganism having N-acetylneuraminic acid aldolase activity and said culture of microorganism capable of producing pyruvic acid or treated matter thereof.

22. (New) The process according to claim 17, wherein said culture of a microorganism carrying DNA encoding N-acetylglucosamine 2-epimerase activity or treated matter thereof is copresent with said cultures of a microorganism having N-acetylneuraminic acid synthetase activity and said culture of microorganism capable of producing phosphoenolpyruvic acid or treated matter thereof.

REMARKS

Claims 4, 5, 17 and 18 have been amended in order to recite the present invention with the specificity required by statute. Additionally, new Claims 19 and 20 are presented in order to more specifically recite various preferred embodiments of the present invention. No new matter has been added.

Claims 1-3 and 6-18 are rejected under 35 U.S.C. §103(a) as being unpatentable over Vanna(a) et al., (Glycobiology, 1997, Vol. 7(5):697-701), or Vann(b) et al., (J. Biol. Chem., 1987, Vol. 262(36):17556-62) and Mura et al., (Carbohydrate